Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1966, 35, 3-15

Mosquito-borne Haemorrhagic Fevers of South and South-East Asia *

SCOTT B. HALSTEAD, M.D.[1]

During the past decade outbreaks of a severe haemorrhagic disease caused by dengue viruses of multiple types have been reported in the Philippines, Thailand, Malaysia, Viet-Nam and eastern India. In many of these outbreaks chikungunya virus, a group A arbovirus, was simultaneously the cause of similar but probably milder disease. Both these viruses appear to be able to produce classical dengue fever in some individuals and disease with haemorrhagic manifestations in others. Because of the growing public health importance and the progressive spread of this disease a unified review of its clinical and epidemiological features has been needed. This paper presents the history and salient clinical features of mosquito-borne haemorrhagic fever and summarizes recent epidemiological studies and current diagnostic and control methods.

Since 1950 arthropod-borne virus haemorrhagic fevers have increased markedly as public health problems. One or more illnesses so named now occur on three continents. Because of the repeated use of the term " haemorrhagic fever " these diseases are commonly thought to be single or related entities, when, in fact, many are readily distinguished by differences in the agent, vector, clinical syndrome or epidemiological pattern. The review of arthropod-borne virus haemorrhagic fevers and proposed classification by vector and agent by Gajdusek (1962) should be consulted for a more complete discussion of this group of diseases.

Of all the arthropod-borne virus haemorrhagic fevers those which are increasing most rapidly as a public health problem occur in South and South-East Asia. These diseases are caused by viruses transmitted by *Aedes aegypti* and are commonly referred to as the mosquito-borne haemorrhagic fevers. The most severe form, associated with dengue viruses of multiple types, is characterized by shock and/or gastrointestinal haemorrhage and frequently death. During some outbreaks chikungunya as well as dengue viruses have simultaneously caused less severe syndromes: classical dengue fever, undifferentiated acute febrile illnesses and milder " haemorrhagic fevers ". The large numbers of viruses which have been identified, the variety of clinical syndromes described and use of different names for the disease in each area have been a cause of considerable confusion. It is the purpose of this review to provide perspective on the problem by summarizing recently acquired knowledge with particular reference to epidemiological and public health features of mosquito-borne haemorrhagic fever.

HISTORY

The term " haemorrhagic fever " was first applied to illness in South-East Asia in the Philippines in 1953 (Hammon, Rudnick et al., 1960). Case reports were first published by Quintos et al. in 1954. Since then, haemorrhagic fever has grown steadily as a disease problem (Fig. 1). In 1956, 1207 patients with Philippine haemorrhagic fever with a mortality rate of 6% were admitted to Manila hospitals.[2] In that year Hammon and his associates began studies which established the dengue etiology of the disease and resulted in the recognition of dengue types 3 and 4 (Hammon, Rudnick & Sather, 1960). It has been

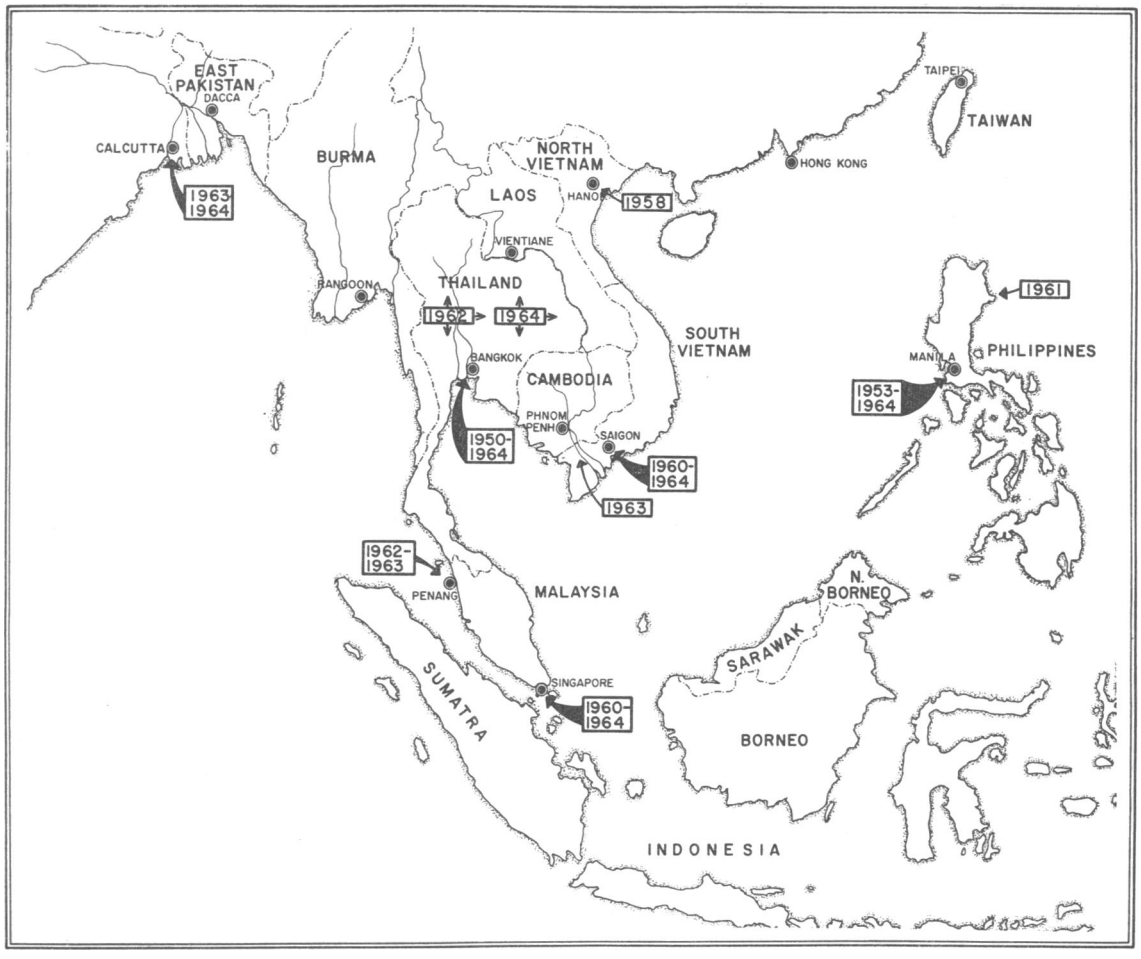
* Presented in part before the Seventh International Congresses on Tropical Medicine and Malaria, Rio de Janeiro, 1-10 September 1963, and The American College of Physicians, Far East Region, Fourth Annual Session, Yokosuka, Japan, 18-20 May 1964. Received for publication in March 1965.

[1] Chief, Virus Department, US Army-SEATO Medical Research Laboratory, Bangkok, Thailand. Present address: Yale Arbovirus Research Unit, 60 College St., New Haven, Conn., USA.

[2] Personal communication from the Disease Intelligence Center, Department of Health, Philippines.

FIG. 1

LOCATION OF MOSQUITO-BORNE HAEMORRHAGIC FEVER OUTBREAKS IN SOUTH AND SOUTH-EAST ASIA, 1953-64



reported since 1956 that 100-500 cases of haemorrhagic fever have been recognized in Philippine hospitals yearly: from March to June 1961, 1160 clinically diagnosed cases were reported from the Isabella region on the island of Luzon.[1]

The presence of haemorrhagic fever in Thailand, although suspected in 1956 following reports of cases in the Philippines, was not identified until 1958, when a sharp outbreak occurred in which nearly 2500 patients were hospitalized with a mortality rate of 10% (Jatanasen, Sakuntanaga & Dhanasiri, 1962). Dengue and chikungunya viruses were recovered from this outbreak (Hammon, Rudnick & Sather, 1960). Of interest in the natural history of the South-East Asian haemorrhagic fevers has been a retrospective report of typical case-histories of Thai haemorrhagic fever in Bangkok each year from 1950 (Halstead & Yamarat, 1965). Between 1950 and 1958 hospitalized cases of a condition resembling haemorrhagic fever have been variously called haemorrhagic influenza with secondary thrombocytopenic purpura, influenza with circulatory failure or Chinese medicine poisoning—the latter so named because of the frequency with which there was an antecedent history of ingestion of an unknown Chinese remedy. In Thailand major outbreaks of haemorrhagic fever have occurred at two-year

[1] Personal communication from the Disease Intelligence Center, Department of Health, Philippines.

intervals with approximately 2500, 1700 and 6000 hospitalizations occurring in 1958, 1960 and 1962, respectively (Jatanasen, Sakuntanaga & Dhanasiri, 1962; Halstead, Yamarat & Scanlon, 1963). Until 1962, haemorrhagic fever was largely confined to the metropolitan area of Bangkok and Thonburi; in that year 2000 cases occurred widely throughout Thailand as far as 400 km north and 700 km south of Bangkok (Halstead, Yamarat & Scanlon, 1963). In every instance disease was limited to population centres in the known areas of distribution of *Aedes aegypti*.

An outbreak of a severe haemorrhagic disease in children in Hanoi, North Viet-Nam, during the rainy season of 1958 has been reported by Mihov, Tuong & Tucng (1959). These authors described clinical features in 68 hospitalized patients which were identical to those seen in virologically proven dengue haemorrhagic fever in other countries. There was a 7% mortality rate in the reported series. Although the exact size of the epidemic is unknown, it was estimated that " many hundreds " of cases occurred in addition to those described. Even in the absence of virological confirmation there appears to be sufficient evidence to include this as an outbreak of mosquito-borne haemorrhagic fever.

In 1960 a non-fatal, dengue-like illness associated frequently with rash, petechiae and thrombocytopenia was observed in Singapore in approximately 200 Asians, predominantly adults (Chew et al., 1961). This illness has received the name Singapore haemorrhagic fever. Since 1962, a severe disease more closely resembling the Thai and Philippine syndromes has been seen with increasing frequency, mainly in children (Lim et al., 1964).

Recently, outbreaks of haemorrhagic fever have been reported in three new areas of South-East Asia. Between November 1962 and April 1964 approximately 61 hospitalized children in Georgetown, Penang, a city in north Malaysia, were diagnosed as suffering from haemorrhagic fever on the basis of clinical and laboratory findings.[1] There have been five deaths. Fourteen viruses isolated from patients are tentatively identified as dengue 2. This outbreak may represent a continuation of the Thai outbreak of 1962, which was known to have extended to a railroad town in Thailand, 700 km south of Bangkok and 150 km north of Penang (Halstead, Yamarat & Scanlon, 1963).

Between June and October 1963, 331 cases of haemorrhagic fever with 116 deaths were recognized in children in South Viet-Namese villages on the Mekong River, 10-50 km south of the Cambodian border. Cases also occurred in the city of Saigon. Dengue 2 viruses have been isolated from patients and mosquitos from this outbreak (Halstead et al., 1965).

Finally, during the months of August through December 1963, a previously unrecognized haemorrhagic disease occurred in Calcutta, India. Records from death registries in the city indicated that at least 158 deaths associated with haemorrhage, vascular collapse or fever may be attributed to the outbreak (Ramakrishnan et al., 1964). The number of hospital cases is unknown. Serological evidence of dengue infection, one dengue type 2 and numerous chikungunya virus isolations have been reported (Shah, Gibbs & Banerjee, 1964; Sarkar, Pavri et al., 1964; Pavri et al., 1964).

CLINICAL MANIFESTATIONS

Little difficulty has been encountered in various haemorrhagic fever outbreaks in recognizing the presence of a severe disease. A unified description of this illness and comparison of clinical features between outbreaks, however, is made difficult for many or all of the following reasons:

(*a*) many descriptions of haemorrhagic fever are based on observations of patients on whom no laboratory virus studies were done and include, therefore, an undetermined number of non-arbovirus infections;

(*b*) clinical terminology, the closeness of patient observation, and the frequency and variety of laboratory studies and of laboratory test methods have not been standardized; and

(*c*) insufficient patients have been etiologically studied to determine whether there is one or many haemorrhagic fever syndromes caused by different types of dengue virus.

A further measure of confusion is added by the observation that two distinct syndromes occur during haemorrhagic fever outbreaks: dengue fever and haemorrhagic fever. Characteristic findings in these two syndromes are summarized in Table 1. Classical dengue fever caused by dengue viruses has been reported infrequently among persons indigenous to dengue endemic areas. However, the dengue fever syndrome regularly occurs in non-indigenous

[1] See summary of the paper by A. Rudnick on page 62 of this issue.

TABLE 1

OBSERVED FREQUENCY [a] OF FINDINGS IN HAEMORRHAGIC FEVER AND IN DENGUE FEVER SYNDROME IN VIROLOGICALLY CONFIRMED DENGUE INFECTIONS, THAILAND, 1962-64

Haemorrhagic fever [b] (357 cases)	Observation	Dengue fever [c] (93 cases)
+	Death	0
+	Shock	0
+	Gastrointestinal bleeding	0
++++	Hepatomegaly	0
++	Leucocytosis	0
+++	Petechiae or ecchymosis	+
++++	Thrombocytopenia	++ [d]
++++	Positive tourniquet test	++ [d]
++++	Fever	++++
+	Leucopenia	++++
+	Generalized lymphadenopathy	++
+	Maculopapular rash	++
+	Myalgia	+++

[a] Frequency of observation: +, 1%-25%; ++, 26%-50%; +++, 51%-75%; ++++, 76%-100%.
[b] Hospitalized patients (mainly Asian children).
[c] Mainly Caucasian adults.
[d] Infrequently tested.

adults and children infected with dengue virus during haemorrhagic fever outbreaks (Halstead & Yamarat, 1965). On the other hand, chikungunya virus may be a cause of classical dengue fever syndrome in both indigenous and non-indigenous persons. This virus is responsible for endemic dengue-like illness in Thailand (Halstead, Yamarat & Scanlon, 1963) and Cambodia (Chastel, 1963), and epidemic disease in India in 1963 (Sarkar, Chatterjee & Chakravarty, 1964) and 1964 (Ramachandra Rao, Carey & Pavri, 1965) and perhaps the "dengue" epidemic in Rangoon in 1963.[1]

As with many infectious diseases, dengue and chikungunya viruses cause a spectrum of illness from mild to severe. An attempt to define these syndromes based upon studies of virologically confirmed patients in Thailand is summarized in Table 2. The following syndrome with and without shock has been described in all countries involved and most logically may be called haemorrhagic fever (Quintos & Lim, 1965; Phitaksphraiwan et al., 1962; Halstead et al., 1965; Mihov, Tuong & Tuong, 1959; Lim, Rudnick & Chan, 1964; Aikat, Konar & Banerjee, 1964).[2]

Disease begins with a febrile or minor illness stage characterized by fever, upper respiratory symptoms, headache, vomiting and abdominal pain. This continues for two to four days, during which time the individual is anorexic but ambulatory and not critically ill.

This stage is followed by an abrupt deterioration in the condition of the patient with the rapid onset of lassitude, weakness and physical collapse. Most patients are brought to the hospital at this time. On examination the child generally has cold, clammy extremities with a warm trunk, flushing of the face, peripheral vascular congestion, restlessness, diaphoresis, and petechiae located most frequently on the forehead and distal extremities and, less frequently, a macular or maculopapular rash. There may be circumoral and extremity cyanosis. The pharynx is injected. Systolic and diastolic blood pressures are low or absent or the differential blood pressure (pulse pressure) may be less than 20 mm Hg. There is marked tachycardia with weak, thready pulse and faint heart sounds. The liver is enlarged two or three finger-breadths, firm and non-tender (infrequent in Philippine cases). There are various changes in normal neurological reflexes and the appearance of abnormal reflexes may be observed.

On the fourth or fifth day of illness the severely ill patient is in danger of dying (Fig. 2). Melaena, haematemesis, coma and deepening or unresponsive shock have a grave prognosis. Following the period of crisis children show steady and fairly rapid improvement.

Common laboratory findings are a positive tourniquest test, prolonged bleeding time, thrombocytopenia, mild leucocytosis with frequent Türk reaction cells, increased haematocrit or haemoglobin concentration and hypoproteinaemia (Cohen & Halstead, 1966). Bone-marrow examination may show maturation arrest of megakaryocytes (Nelson, Bierman & Chulajata, 1964). X-ray evidence of right-sided pleural effusion and pneumonitis is frequent (Nelson, 1960). Abnormalities in liver chemistries parallel disease severity.[3] There may be

[1] Unpublished observation by the author.

[2] See summary of the paper by N. Paramaesvaran on page 40 of this issue.

[3] See summary of the paper by M. Balankura et al. on page 51 of this issue.

TABLE 2

CLINICAL SYNDROMES CAUSED BY DENGUE AND CHIKUNGUNYA VIRUSES IN THAILAND [a]

Syndrome	Findings	Virus etiology	Prognosis
Acute undifferentiated respiratory disease	Fever, coryza, pharyngeal inflammation with or without cough.	Dengue, chikungunya	Good
Undifferentiated fever	Fever with or without symptoms of multiple system involvement.	Dengue, chikungunya	Good
Dengue fever	Fever, myalgia and/or arthralgia and leucopenia with or without rash, headache, lymphadenopathy, biphasic fever, nausea, vomiting, positive tourniquet test, scattered petechiae and thrombocytopenia.	Dengue, chikungunya	Good
Haemorrhagic fever without shock	Undifferentiated fever for 2 or more days followed by two or more of the following: petechiae, purpura, ecchymosis, epistaxis, positive tourniquet test, thrombocytopenia, hepatomegaly.	Dengue, occasionally chikungunya [b]	Good
Haemorrhagic fever with shock	Same as above except accompanied by shock (absent systolic and/or diastolic blood pressure, pulse pressure 20 mm Hg or less) and/or severe gastrointestinal haemorrhage.	Dengue	30%-50% mortality

[a] Unpublished data of S. B. Halstead and S. Nimmannitya.

[b] In Thai children symptoms of chikungunya infections are intermediate between dengue fever and haemorrhagic fever and are characterized by sudden onset of high fever with positive tourniquet test, petechiae and rash.

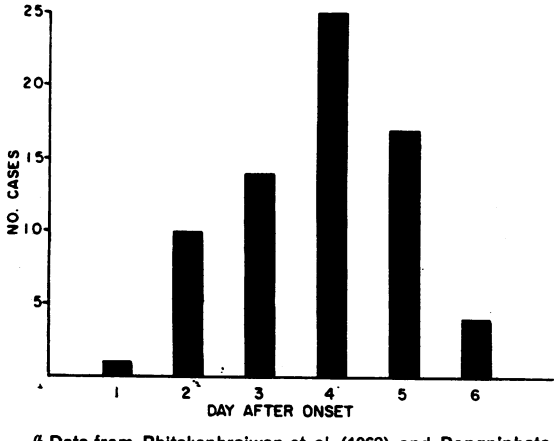
evidence of diffuse myocardial abnormality on electrocardiogram (Toochinda, 1962).

PATHOLOGY

Because virus isolations from autopsy organs in mosquito-borne haemorrhagic fever are extremely rare, the reported pathological studies of this disease are on patients identified only clinically and epidemiologically (Bhamarapravati, 1962; De et al., 1964). There are no pathognomonic, gross or microscopic findings. On gross examination focal or subserosal haemorrhage, gastrointestinal haemorrhage, tissue oedema, clear or blood-tinged fluid in serosal cavities and patchy areas of pneumonitis are frequently found. Microscopically, the most commonly reported findings are maturation arrest of megakaryocytes or hypocellularity of bone marrow, focal areas of hepatic necrosis, infiltration of portal areas and sinusoids by lymphoid cells, marked increase of lymphyocytoid and plasmacytoid cells, increase in phagocytic activity and interstitial pneumonitis. Involvement of the kidneys, adrenal, pituitary or central nervous system is rare.

FIG. 2

DAY OF DEATH AFTER ONSET OF THAI HAEMORRHAGIC FEVER IN 66 PATIENTS AT CHULALONGKORN AND CHILDREN'S HOSPITALS, BANGKOK, 1958-60 [a]



[a] Data from Phitaksphraiwan et al. (1962) and Pongpiphata (1962).

ETIOLOGY

If etiological consideration is restricted to severe haemorrhagic fever—i.e., disease characterized by shock, and/or gastrointestinal haemorrhage or death—only a few virus isolations have been reported and these from Thailand. As shown in Table 3, most viruses recovered from severe haemorrhagic fever are identical or closely related to dengue types 1 and 2 (in this paper viruses of the TH-Sman-

TABLE 3

VIRUSES ASSOCIATED WITH SEVERE HAEMORRHAGIC FEVER OR DEATH IN SOUTH AND SOUTH-EAST ASIA 1956-63

Country and author	Disease severity	
	Haemorrhagic fever with shock and/or gastrointestinal haemorrhage [a]	Death [a]
Philippines		
Hammon, Rudnick & Sather (1960)	Dengue isolated.	Dengue isolated.
Viet-Nam		
Halstead et al. (1965)	Dengue isolated.	Dengue isolated.
Cambodia		
Chastel (1963)	Dengue and chikungunya isolated; no haemorrhagic fever reported.	
Thailand		
Dasaneyavaja, Robin & Yenbutra (1963)	Dengue 2 isolated in 3/61 patients.	Dengue 4 isolated from liver tissue in 1/? fatal cases.
Halstead—unpublished	Dengue 1 isolated in 2/74 patients.	Dengue 1 isolated from acute serum or heart blood in 1/72 fatal cases.
	Dengue 2 isolated in 8/74 patients.	Dengue 2 isolated from acute serum or heart blood in 3/72 fatal cases.
Malaysia		
Lim et al. (1964)	Dengue isolated.	Dengue isolated.
India		
Sarkar, Chatterjee et al. (1965)	Dengue isolated; chikungunya isolations reported from five patients with haemorrhage and/or shock (undefined).[b]	Chikungunya isolated from acute serum or heart blood in 2/? fatal cases.

[a] " Dengue isolated " indicates dengue virus isolation or serological confirmation related in time or place to severe disease.
[b] Non-haemorrhagic illness resembling that seen in Thailand has been described in 33 virologically confirmed chikungunya infections in children in Vellore, India, in 1964 (Jadhav et al., 1965).

dengue-1 group and viruses of the TH-36–dengue-2 group are referred to as dengue 1 and dengue 2, respectively). In other outbreaks the etiology of severe haemorrhagic fever may only be surmised since clinical descriptions of patients with virus isolations are not included in published accounts. In the Penang, Calcutta and South Viet-Nam outbreaks in which severe haemorrhagic fever with fatalities were reported only dengue type 2 viruses were recovered from humans (Sarkar, Pavri et al., 1964; Halstead et al., 1965).[1] Serological responses in larger numbers of patients in these outbreaks are also compatible with recent dengue 2 infection. Although dengue types 3 and 4 have been recovered from patients during haemorrhagic fever outbreaks in the Philippines, Thailand and Singapore, direct or frequent association of these virus types with severe haemorrhagic fever has not yet been established (Hammon, Rudnick & Sather, 1960; Dasaneyavaja, Robin & Yenbutra, 1963; Lim et al., 1964). It may be pertinent that dengue 2 virus was recovered once from a patient during the 1956 Philippine outbreak and that serological studies did not exclude the possibility that dengue 2 was the cause of severe infection (Hammon, Rudnick & Sather, 1960).

At least four, and possibly six, types of dengue and chikungunya virus have been isolated from arthropods and man during haemorrhagic fever or

[1] See summary of the paper by A. Rudnick on page 62 of this issue.

TABLE 4

DENGUE AND CHIKUNGUNYA VIRUSES ISOLATED FROM SOUTH AND SOUTH-EAST ASIA, 1956-63

Source of isolation	Philippines	Viet-Nam	Cambodia	Thailand	Singapore	Malaysia (Penang)	India	
							Calcutta	Vellore
Man			Dengue 1 [a]	Dengue 1 [b, c, d]	Dengue 1 [e]			Dengue 1 [f]
	Dengue 2 [b]	Dengue 2 [g]		Dengue 2 [b, c, h, i]	Dengue 2 [e]	Dengue 2 [j]	Dengue 2 [k]	Dengue 2 [f]
	Dengue 3 [b]			Dengue 3 [c]	Dengue 3 [l]			
	Dengue 4 [b]		Dengue 4 [a]	Dengue 4 [h, m]	Dengue 4 [n]			Dengue 4 [f]
			Chikungunya [o]	Chikungunya [b, h, p]			Chikungunya [k]	
Aedes aegypti				Dengue 1 [c, d]				Dengue 1 [f]
		Dengue 2 [g]		Dengue 2 [b, c, i]				
	Dengue 3 [b]			Dengue 3 [c]				
				Dengue 4 [c]				Dengue 4
				Chikungunya [p]			Chikungunya [q]	
Culex quinquefasciatus				Chikungunya [p, r, s]				
Culex tritaeniorhynchus	Dengue 3 [b]							

[a] Chastel—see page 84 of this issue.
[b] Hammon, Rudnick & Sather (1960).
[c] Halstead—unpublished data.
[d] Antigenic variation of some strains may be sufficient to rename as type 6.
[e] Lim, Rudnick & Chan (1961).
[f] Carey, Meyers & Reuben (1964).
[g] Halstead et al. (1965).
[h] Dasaneyavaja & Pongsupat (1961).
Antigenic variation of some strains may be sufficient to rename as type 5.
[j] Rudnick—see page 62 of this issue.
[k] Sarkar, Pavri et al. (1964).
[l] Chan—see page 61 of this issue.
[m] Dasaneyavaja, Robin & Yenbutra (1963).
[n] Lim, Chan et al. (1964).
[o] Chastel (1963).
[p] Halstead, Yamarat & Scanlon (1963).
[q] Pavri et al. (1964).
[r] Robin, Yenbutra & Dasaneyavaja (1963).
[s] Hammon & Sather (1962).

dengue fever outbreaks in South-East Asia. The distribution of these viruses shown in Table 4 probably reflects the amount and duration of virological studies rather more accurately than it does the actual virus transmission during the period covered.

EPIDEMIOLOGY

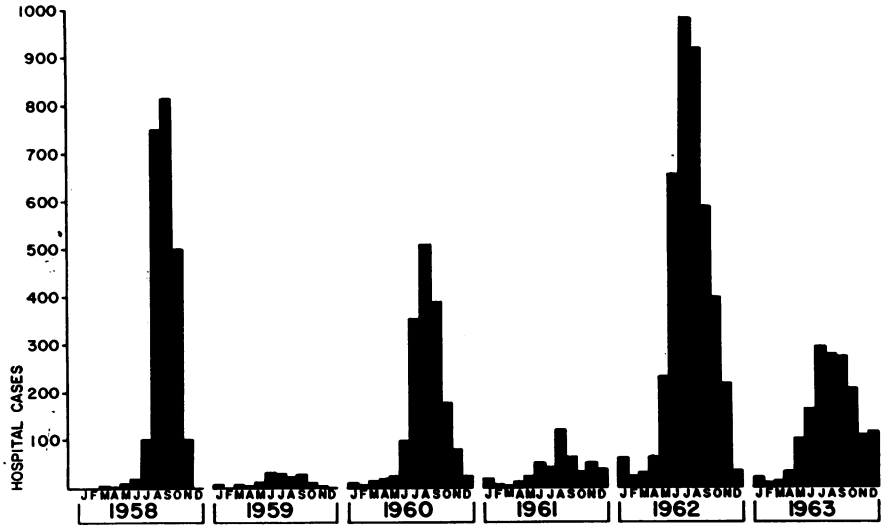
Many problems remain in understanding the epidemiological phenomena of haemorrhagic fever. The disease has become a major problem in areas where there is evidence that the dengue fever syndrome had been endemic for many years. Outbreaks, since recognition, have tended to recur at the original site and spread to other *Aedes aegypti*-infested areas within the country. At the same time urban centres of Indonesia, Cambodia, Burma, East Pakistan and South India have not reported haemorrhagic fever. The reason for this patchy international occurrence is obscure, as are the reasons for the sudden " appearance " of this disease in the past decade.

Observations of the haemorrhagic fever syndrome (including non-shock cases) in Thailand may serve to illustrate epidemiological aspects common to other outbreaks in urban areas at similar latitudes.

Haemorrhagic fever is seasonal and recurrent. Fig. 3 shows the monthly disease occurrence from 1958 to 1963 in the Bangkok-Thonburi urban area. It can be seen that outbreaks are confined to the rainy season (May to November) and that the disease has occurred every year with larger epidemics in alternate years.

Haemorrhagic fever is a children's disease. Of the 10 367 cases, with 694 deaths, among Bangkok-Thonburi residents from 1958 to 1963, all but 25 cases were in children under the age of 14. Disease attack

FIG. 3
MONTHLY HOSPITALIZATIONS FOR THAI HAEMORRHAGIC FEVER IN BANGKOK AND THONBURI, THAILAND, 1958-63



rates were nearly identical in males and females of any age. No consistent variation in mortality was observed in age-groups with large numbers of cases.

Haemorrhagic fever attack rates in various ethnic and socio-economic groups have been the subject of investigation in 1962.[1] While hospitalization rates were somewhat low in the extremely high economic class, there was little difference in disease occurrence or severity throughout the rest of the social strata. Disease rates in Thai and Chinese ethnic groups were directly proportional to their representation in the Bangkok-Thonburi population.

In the 1962 study (Halstead, Yamarat & Scanlon, 1963) hospital admissions reflected only a portion of the total morbidity due to haemorrhagic fever viruses. On the basis of a house-to-house random sample of 3% of the Bangkok population, it was estimated that 4000 children in addition to those hospitalized (4187) were diagnosed as having haemorrhagic fever without shock by physicians in Bangkok and were treated in private clinics or at home. Minor illness associated with the epidemic occurred at an enormous rate. A study of febrile illness in out-patients conducted throughout the haemorrhagic fever season at the Children's Hospital in Bangkok has shown that approximately 17% (or 13 000) of the total patients seen in the out-patient department and not admitted to the hospital in 1962 had infections with dengue or chikungunya viruses. If this figure is applied to the total paediatric out-patient visits for all hospitals in Bangkok and Thonburi, there would have been an estimated 78 000 children with minor illness due to dengue and chikungunya viruses who received medical attention at hospitals alone. Up to twice this number of children with minor illness may have been seen by practising physicians. This would mean that in Bangkok and Thonburi there were an estimated 150 000 to 200 000 minor illnesses in children under the age of 15 in a total population of 870 000 for this age-group! The over-all haemagglutination-inhibiting antibody conversion rates for chikungunya and dengue viruses calculated from a sample of over 2000 persons bled before and after the outbreak was approximately 30% each. In brief, the impact of dengue and chikungunya viruses on the population of Bangkok in 1962 was tremendous.

[1] See summary of the paper by S. B. Halstead on page 80 of this issue.

ARTHROPOD VECTORS AND VERTEBRATE HOSTS

Aedes aegypti has been incriminated as the vector of both chikungunya and dengue viruses on the basis of epidemiological, virus isolation and virus transmission studies. *Aedes aegypti* in South-East Asia is highly anthropophilic and breeds in artificial con-

FIG. 4
TYPICAL SHANGHAI JAR (*ONG*) USED FOR WATER STORAGE AND A MAJOR BREEDING-SITE OF *AEDES AEGYPTI*

tainers and receptacles. The cultural practice of collection and storage of water in South-East Asia provides abundant breeding areas for *Aedes aegypti* throughout the year (Fig. 4). In the rainy season these and other containers are filled with fresh water and *Aedes aegypti* prospers. In Thailand peak *Aedes aegypti* populations occur just after the first rains in May.[1] Generally, the epidemic peak follows two months later. In areas without marked seasonal rainfall cases may occur throughout the year.

Virus recoveries from mosquitos have been summarized in Table 4. All dengue virus types and chikungunya virus have been recovered repeatedly from *Aedes aegypti*. In a longitudinal study of Thai haemorrhagic fever in five sites in Bangkok, conducted in 1962, 27 viruses were recovered from 150 pools of *Aedes aegypti* (Halstead, Yamarat & Scanlon, 1963). Seven of the isolates were chikungunya virus and 20 were dengue viruses types 1-4. Isolations of chikungunya virus from *Culex pipiens quinquefasciatus* (=*C.p. fatigans*) have also been reported (Hammon & Sather, 1962; Robin, Yenbutra & Dasaneyavaja, 1963). Among 753 pools representing 79 453 mosquitos of this species collected in Bangkok in 1962, only one isolation of chikungunya virus was made (Halstead, Yamarat & Scanlon, 1963). Successful chikungunya transmission studies have been reported using *Aedes aegypti* and *Aedes albopictus* (McIntosh et al., 1963; Ramachandra Rao, Singh & Pavri, 1964; Shah, Gilotra et al., 1964) but the same authors have failed to demonstrate chikungunya virus survival in or transmission by *Culex pipiens quinquefasciatus*. It seems unlikely that this latter species is an important vector of chikungunya, although infrequent recoveries reported from *C. quinquefasciatus* imply the possibility of occasional mechanical transmission.

While the man–*Aedes-aegypti*–man cycle of dengue is undoubtedly responsible for urban epidemics, it has been suggested that a sylvan cycle involving monkeys and *Aedes albopictus* may exist in the tropics (Simmons, St. John & Reynolds, 1931). Recent isolates of chikungunya from *Culex tritaeniorhynchus* in areas of Thailand where human population densities were low suggests a non-human virus reservoir for this virus.[2] Antibody surveys in Thailand have shown chikungunya haemagglutination-inhibiting antibodies (Hammon, Sather & Rudnick, 1962b) and neutralizing antibodies in large domestic animals.[2]

LABORATORY DIAGNOSIS

Specific virological diagnosis of dengue virus infection from study of antibody is frequently difficult. This is often due to a broad group B response detected by haemagglutination-inhibition (HI), complement-fixation (CF) and neutralization tests (Hammon & Sather, 1964b). This broad reaction may be caused by preceding infection with group B arboviruses, in most instances other dengue viruses. Occasionally, however, individuals previously unexposed to dengue virus also show a multivalent antibody response in convalescent sera.

It has been suggested that antibody response to primary and secondary dengue virus infection may be differentiated by the rate of HI and CF antibody formation and the development of CF antibody to non-dengue group B arboviruses (Hammon & Sather, 1964a). In the secondary response CF antibody broadly reactive to group B is frequently found in the acute phase or early convalescent serum. In primary dengue infection relatively specific CF antibody is detected often only after the 14th post-infection day.

[1] See summary of the paper by J. E. Scanlon on page 81 of this issue.

[2] See summary of the paper by S. B. Halstead & S. Udomsakdi on page 89 of this issue.

Experience with the neutralization test with dengue viruses is not yet extensive. Neutralization tests may be done in suckling or weanling mice, tube cell cultures or by use of plaque techniques. Most experience to date has been with the virus dilution technique. Further study of serum dilution and plaque reduction techniques are required before the utility of the neutralization test as a diagnostic technique is known.

Since multiple dengue infections are common in most haemorrhagic fever areas, virus isolation provides the only means for identification of the specific viral agent. The suckling mouse is the most widely employed host for dengue virus recovery. There is evidence that all mouse strains are not equally satisfactory for intracerebral adaptation of dengue viruses. Such a conclusion would explain the reported differences in dengue virus isolation experience in mice. Some laboratories have reported difficulty in obtaining high-titred, uniformly lethal, dengue virus preparations (Hammon & Sather, 1962), while others have readily adapted dengue viruses to mice after few passages (Lim et al., 1964). One of the methods used successfully is the dengue challenge technique, in which mice are inoculated with suspect material and after 21-28 days challenged by inoculating with 100 LD_{50} of weanling mouse lethal dengue virus intracerebrally. Litter mates of mice surviving challenge are blind-passaged for recovery of virus (Hammon & Sather, 1964a). Routine blind passage at 10-day intervals has also been employed for recovery and quick adaptation of dengue viruses (Dasaneyavaja & Pongsupat, 1961).

Recently, tissue cultures have been used for recovery of dengue viruses (Halstead, Sukhavachana & Nisalak, 1964a). Although dengue viruses do not readily produce cytopathogenic effect in mammalian cells, their presence may be detected by the development of resistance of dengue-infected cells to other cytopathogenic viruses (Halstead, Sukhavachana & Nisalak, 1964b). Plaque methods now being developed promise additional versatility in study of dengue viruses.[1]

Once isolated, dengue viruses must be identified by serological methods. It is not known yet whether dengue viruses from haemorrhagic fever endemic areas are antigenically identical with those from other areas. It has been suggested that viruses related to types 1 and 2 but distinct from them are prevalent in South-East Asia (Hammon & Sather, 1964b).

Chikungunya virus diagnosis causes less difficulty in the laboratory. The virus is readily recovered in suckling mice or hamster kidney cells from acute sera. " Auto-interference ", which develops in low suckling-mouse-passsage brain preparations, may be avoided by making all virus passages at 10^{-3} or higher dilution. Fourfold antibody response is usually readily demonstrated in paired sera as HI antibody develops late in the course of illness. CF antibody response is frequently delayed until two weeks or more after onset of symptoms.[2] Neutralization tests may be done in mice, in a variety of cell cultures or with plaque methods. The warning must be made that chikungunya virus is stable under laboratory conditions, easily aerosolized and infectious to man by the respiratory route. Infection of susceptible laboratory workers has occurred in a number of laboratories (McIntosh et al., 1963).[3]

CONTROL

Although vaccination is a theoretical method of control of dengue and chikungunya epidemics, development of vaccines for these viruses has been slow. Since dengue viruses are generally considered to be poorly antigenic, current efforts centre on attenuated live virus vaccine. Low mouse-passage attenuated virus vaccines are available for dengue types 1 and 2 (Sabin, 1959; Wisseman et al., 1963). These vaccines have been shown to protect against homologous challenge and provide heterologous protection for several months. Experiments with multivalent vaccine and sequential parenteral vaccination with types 1 and 2 at six-week intervals have failed to confer protection to both types (Sabin, 1959). Thus, vaccination with four different dengue virus types can be expected to be a long process as it will be necessary to wait until heterologous antibody disappears to ensure vaccine " takes ". A living or killed virus vaccine for chikungunya viruses would appear to present few of these difficulties.

In the absence of a suitable vaccine, vector control is the only method available to combat haemorrhagic fever. Areas which are infested by *Aedes aegypti* but free of dengue or chikungunya viruses would be well

[1] See summary of the paper by P. Sukhavachana et al. on page 65 of this issue.

[2] See summary of the paper by S. Udomsakdi et al. on page 68 of this issue.

[3] Also, unpublished observations of S. B. Halstead (3 cases), M. Kitoaka (6 cases) and F. M. Cadigan (4 cases).

advised to adopt quarantine procedures or case-finding techniques for passengers arriving from haemorrhagic fever epidemic areas.

Permanent control of *Aedes aegypti* must combine public co-operation in reducing household breeding-sites with provision of reliable piped water to all residences and the destruction of breeding-sites outside houses. For the immediate future such measures must be supplemented by a carefully planned *Aedes aegypti* control programme using insecticides against larvae and adults as well as the full range of public health measures. It has been strongly recommended that control measures be instituted immediately in highly endemic urban areas to reduce transmission of viruses and thereby prevent the seeding of satellite outbreaks.[1]

DISCUSSION

The association of dengue and chikungunya viruses in time and place with severe haemorrhagic disease has led many authors to assume that both viruses cause "haemorrhagic fever" (Hammon, Rudnick & Sather, 1960; Halstead & Buescher, 1961; Chastel, 1963; Miles, 1964). As was first suggested by Dasaneyavaja, Robin & Yenbutra (1963) and confirmed in a large series studied by the SEATO Medical Research Laboratory and the Children's Hospital, Bangkok,[2] the most severe form of chikungunya infection observed in Thailand has been a febrile illness with minor haemorrhagic manifestations (haemorrhagic fever without shock) and not a life-threatening disease. If uniform clinical criteria and diagnostic terminology are adopted as recommended elsewhere in this issue,[1] future evaluation of various syndromes caused by chikungunya and dengue viruses should be facilitated. Further, the use of clinical diagnosis irrespective of etiology should end the unfortunate tendency to confuse diagnosis and etiology, e.g., diagnosing as haemorrhagic fever a non-specific febrile disease confirmed in the laboratory as dengue infection.

It is difficult to understand why dengue viruses, usually benign, now cause severe and frequently fatal illness. It should be recalled, however, that illnesses characterized by haemorrhage or shock and often with fatal outcome have accompanied classical dengue fever outbreaks perhaps five times in the past 70 years: in North Queensland, Australia, in 1897 (Hare, 1898); in the southern USA in 1922 (Scott, 1923); in South Africa in 1927 (*Monthly epidem. Rep.*, 1928); in Greece in 1928 (Copanaris, 1928); and in Taiwan in 1931 (Nomura & Akashi, 1931). Each of these outbreaks was self-limited. Despite the lack of direct evidence that these haemorrhagic illnesses were caused by dengue viruses, the high frequency of association of the two diseases supports the hypothesis that dengue viruses have previously acquired pathological properties. From the public health point of view there is little alternative to assuming that dengue is mutable and that variants cause haemorrhagic fever. Dengue viruses in South and South-East Asia must be considered dangerously pathogenic until proved otherwise.

Many observations suggest that host factors play a role in the pathogenesis of severe manifestations of dengue infection. Some of the possibilities of host-controlled disease mechanisms have been discussed elsewhere (Halstead & Yamarat, 1965). Briefly, the association of haemorrhagic fever in areas where multiple dengue viruses simultaneously cause disease, the occurrence of only the classical dengue fever syndrome in short-term residents of South and South-East Asia and the accelerated or secondary type of antibody response observed frequently in haemorrhagic fever patients have led to the hypothesis that haemorrhagic fever may be a kind of hyperimmune response in individuals sensitized by previous dengue infection. The assumption that genetic factors in these populations may be at least partly responsible for the severe dengue virus syndrome has been postulated because of the limitation thus far of mosquito-borne haemorrhagic fever to South Asian people.

As is required for other ubiquitous viral agents, rigid laboratory criteria must be observed in establishing clinical and etiological associations in dengue and chikungunya disease. When two or more arboviruses are being transmitted simultaneously, isolation of virus from serum or autopsy materials alone is not sufficient to establish etiology. Infection with other viruses should be excluded or antibody rise to the recovered virus demonstrated. The widely employed technique of blind passage of suspect materials plus the relative stability of dengue and, particularly, of chikungunya virus make false positive isolations a constant threat. Reisolation of agents from separate aliquots of original material is also important in establishing reliable data.

Whether all dengue viruses are equally pathogenic has not yet been established. The best evidence

[1] See the memorandum on page 17 of this issue.

[2] See summary of the paper by S. Nimmannitya & P. Mansuwan on page 42 of this issue.

supports the view that dengue 2 (or related viruses) plays a role in severe disease. Several type 1 viruses have also been recovered from severely ill children. More study will be required to define the pathogenicity of dengue types 3 and 4. The isolation of dengue 4 from human liver reported by Dasaneyavaja, Robin & Yenbutra (1963) is in contradiction to other experience in Thailand, in which no dengue virus recoveries of any type were made from tissues of nearly 60 patients.[1] If host factors determine disease outcome, then any dengue virus may be pathogenic. The accelerating pace of research on this disease provides hope that answers to these and other questions will be available in the near future.

[1] See summary of the paper by P. Singharaj et al. on page 57 of this issue.

RÉSUMÉ

Au cours des quinze dernières années, des épidémies de maladies fébriles hémorragiques se sont produites aux Philippines, en Malaisie, en Thaïlande, au Viet-Nam et en Inde orientale. Dans les formes sévères, la maladie débute par une période fébrile de plusieurs jours; une aggravation soudaine avec choc, collapsus cardio-vasculaire, éruption pétéchiale et manifestations hémorragiques, peut entraîner la mort au 4e-5e jour, sinon la guérison est rapide. En Thaïlande et aux Philippines, la mortalité a atteint 10% et 5% des cas hospitalisés, par melaena, hématémèse, coma et choc. Les malades avaient présenté une thrombocytopénie, leucocytose modérée avec présence fréquente de cellules de Türk, augmentation du volume globulaire relatif et de la concentration en hémoglobine; dans la moelle, la maturation des mégacaryocytes était arrêtée; les atteintes du foie étaient communes. Les virus isolés, chez des malades et des moustiques, au cours de ces poussées épidémiques, appartenaient au groupe de la dengue et au groupe A des arbovirus: aux Philippines, dengue des types 2, 3 et 4; en Thaïlande, dengue des types 1, 2 et 3; à Singapour, dengue des types 1, 2, 3 et 4; en Thaïlande et en Inde, virus Chikungunya.

La maladie est devenue un problème grave dans des régions où la dengue est endémique depuis de nombreuses années. Elle a réapparu aux mêmes endroits par poussées annuelles, plus fortes tous les deux ans, et tend à s'étendre vers d'autres régions infestées par *Aedes aegypti*; les poussées épidémiques se produisent au cours de la saison des pluies. Les virus ont été isolés principalement de *A. aegypti* mais aussi de *Culex pipiens fatigans* et d'autres moustiques. Il est difficile de comprendre pourquoi les virus de la dengue, habituellement peu pathogènes, provoquent maintenant une maladie grave, souvent mortelle. Cependant, dans le passé, au moins cinq épidémies de dengue classique se sont accompagnées d'une maladie, avec hémorragies ou phénomènes de choc, souvent fatale. La stabilité des virus de la dengue ne permet pas de supposer que des mutants soient responsables des fièvres hémorragiques; on peut admettre que ceux de l'Asie du Sud et du Sud-Est ont un pouvoir pathogène dangereux. Mais l'existence de virus de la dengue dans les régions touchées par ces fièvres hémorragiques, le fait que les personnes qui y résident temporairement présentent des atteintes de dengue classiques, la formation accélérée des anticorps au cours des fièvres hémorragiques, font supposer que ces dernières sont une réponse d'hyperimmunité d'individus sensibilisés par une atteinte antérieure de dengue. Le rôle de facteurs génétiques a aussi été avancé.

L'auteur trouve que le terme de fièvre hémorragique transmise par les moustiques est acceptable. Il étudie les moyens de prévention: vaccins vivants atténués — les virus de la dengue étant de médiocres antigènes — et surtout lutte contre les vecteurs, principalement *A. aegypti*. Ces mesures sont nécessaires en raison de l'importance croissante des épidémies.

REFERENCES

Aikat, B. K., Konor, N. R. & Banerjee, G. (1964) *Indian J. med. Res.*, **52**, 660-675

Bhamarapravati, N. (1962) *The spectrum of pathological changes in Thai haemorrhagic fever.* In: *Symposium on Haemorrhagic Fever, 1961*, Bangkok, pp. 76-87 (SEATO Medical Research Monograph No. 2)

Carey, D. E., Myers, R. M. & Reuben, R. (1964) *Science*, **143**, 131-132

Chastel, C. (1963) *Bull. Soc. Path. exot.*, **56**, 892-915

Chew, A., Gwee, A. L., Ho, Y., Khoo, O. T., Lee, Y. K., Lim, C. H. & Wells, R. (1961) *Lancet*, **1**, 307-310

Cohen, S. N. & Halstead, S. B. (1966) *J. Pediat.*, **68**, 448-456

Copanaris, P. (1928) *Bull. Off. Int. Hyg. publ.*, **20**, 1590-1601

Dasaneyavaja, A. & Pongsupat, S. (1961) *J. trop. Med. Hyg.*, **64**, 310-314

Dasaneyavaja, A., Robin, Y. & Yenbutra, D. (1963) *J. trop. Med. Hyg.*, **66**, 35-41

De, S. N., Chowdhury, B., Sen, S. N., Mukherjee, S. & Venkatachalam, M. A. (1964) *J. Indian med. Ass.*, **42**, 557-560

Gajdusek, D. C. (1962) *J. Pediat.*, **60**, 841-857
Halstead, S. B. & Buescher, E. L. (1961) *Science*, **134**, 475-476
Halstead, S. B., Sukhavachana, P. & Nisalak, A. (1964a) *Nature (Lond.)*, **202**, 931-932
Halstead, S. B., Sukhavachana, P. & Nisalak, A. (1964b) *Proc. Soc. exp. Biol. (N.Y.)*, **115**, 1062-1068
Halstead, S. B., Voulgaropoulos, E., Tien, N. H. & Udomsakdi, S. (1965) *Amer. J. trop. Med. Hyg.*, **14**, 819-830
Halstead, S. B. & Yamarat, C. (1965) *Amer. J. publ. Hlth*, **55**, 1386-1395
Halstead, S. B., Yamarat, C. & Scanlon, J. E. (1963) *J. med. Ass. Thailand*, **46**, 449-462
Hammon, W. M., Rudnick, A. & Sather, G. E. (1960) *Science*, **131**, 1102-1103
Hammon, W. M., Rudnick, A., Sather, G. E., Rogers, K. D. & Morse, L. J. (1960) *Trans. Ass. Amer. Phycns*, **73**, 140-155
Hammon, W. M. & Sather, G. E. (1962) *Chikungunya and unidentified viruses from Thai haemorrhagic fever cases.* In: *Symposium on Haemorrhagic Fever, 1961*, Bangkok, p. 39-41 (SEATO Medical Research Monograph No. 2)
Hammon, W. M. & Sather, G. E. (1964a) *Amer. J. trop. Med. Hyg.*, **13**, 629-641
Hammon, W. M. & Sather, G. E. (1964b) *Milit. Med.*, **129**, 130-135
Hammon, W. M., Sather, G. E. & Rudnick, A. (1962a) *Identification and classification of the dengue group of viruses.* In: *Symposium on Haemorrhagic Fever, 1961*, Bangkok, pp. 30-36 (SEATO Medical Research Monograph No. 2)
Hammon, W. M. Sather, G. E. & Rudnick, A. (1962b) *Serology of haemorrhagic fever patients in the Philippines (1956), in Bangkok (1958, 1960) and in normal children and animals in Bangkok (1958).* In: *Symposium on Haemorrhagic Fever, 1961*, Bangkok, pp. 45-58 (SEATO Medical Research Monograph No. 2)
Hare, F. E. (1898) *Australas. med. Gaz.*, **17**, 98-107
Jadhav, M., Namboodripad, M., Carman, R. H., Carey, D. E. & Meyers, R. M. (1965) *Indian J. med. Res.*, **53**, 764-776
Jatanasen, S., Sakuntanaga, P. & Dhanasiri, C. (1962) *Some aspects of epidemiology of Thai haemorrhagic fever, 1958-1961.* In: *Symposium on Haemorrhagic Fever, 1961*, Bangkok, pp. 6-18 (SEATO Medical Research Monograph No. 2)
Lim, K. A., Chan, Y. C., Phoon, W. O. & Hanam, E. (1964) *Bull. Wld Hlth Org.*, **30**, 227-240
Lim, K. A., Rudnick, A. & Chan, Y. C. (1961) *Singapore med. J.*, **2**, 158-161
McIntosh, B. M., Paterson, H. E., Donaldson, J. M. & De Sousa, J. (1963) *S. Afr. J. med. Sci.*, **28**, 45-52
Mihov, C., Tuong, C. V. & Tuong, H. P. (1959) *Folia med. (Sofia)*, **1**, 169-173
Monthly epidem. Rep., 1928, **7**, 334-338
Nelson, E. R. (1960) *J. Pediat.*, **56**, 101-108
Nelson, E. R., Bierman, H. R. & Chulajata, R. (1964) *Amer. J. trop. med. Hyg.*, **13**, 642-649
Nomura, S. & Akashi, K. (1931) *Taiwan Igakkai Zasshi*, **30**, 1154-1157
Pavri, K. M., Banerjee, G., Anderson, C. R. & Aikat, B. K. (1964) *Indian J. med. Res.*, **52**, 692-697
Phitaksphraiwan, P., Nimmannitya, S., Rajvejpisan, V. & Prasatvinichai, S. (1962) *Clinical observations on Thai haemorrhagic fever in the Children's Hospital (1958-1960).* In: *Symposium on Haemorrhagic Fever, 1961*, Bangkok, pp. 88-100 (SEATO Medical Research Monograph No. 2)
Piyaratn, P. (1961) *Amer. J. trop. Med. Hyg.*, **10**, 767-772
Pongpiphata, S. (1962) *Clinical symptomatology of Thai haemorrhagic fever . . .* In: *Symposium on Haemorrhagic Fever, 1961*, Bangkok, pp. 111-116 (SEATO Medical Research Monograph No. 2)
Quintos, F. N., & Lim, L. E. (1956) *S. Tomás J. Med.*, **11**, 319-328
Quintos, F. N., Lim, L., Juliano, L., Reyes, A. & Lacson, P. (1954) *Philipp. J. Pediat.*, **3**, 1-19
Ramachandra Rao, T., Carey, D. E. & Pavri, K. M. (1965) *Indian J. med. Res.*, **53**, 689-693
Ramachandra Rao, T., Singh, K.R.P. & Pavri, K. M. (1964) *Curr. Sci.*, **33**, 235-236
Ramakrishnan, S. P., Gelfand, H. M., Bose, P. N., Sehgal, P. N. & Mukherjee, R. N. (1964) *Indian J. med. Res.*, **52**, 633-650
Robin, Y., Yenbutra, D. & Dasaneyavaja, A. (1963) *Méd. trop.*, **23**, 781-787
Sabin, A. B. (1959) In: Rivers, T. M. & Horsfall, F. L., Jr, ed., *Viral and rickettsial diseases of man*, 3rd ed., Philadelphia, Lippincott, p. 371
Sarkar, J. K., Chatterjee, S. N. & Chakravarty, S. K. (1964) *Indian J. med. Res.*, **52**, 651-659
Sarkar, J. K., Chatterjee, S. N., Chakravarty, S. K. & Mitra, A. C. (1965) *Indian J. med. Res.*, **53**, 921-925
Sarkar, J. K., Pavri, K. M., Chatterjee, S. N., Chakravarty, S. K. & Anderson, C. R. (1964) *Indian J. med. Res.*, **52**, 684-691
Scott, L. C. (1923) *J. Amer. med. Ass.*, **80**, 387-393
Shah, K. V., Gibbs, C. J., Jr & Banerjee, G. (1964) *Indian J. med. Res.*, **52**, 676-683
Shah, K. V., Gilotra, S. K., Gibbs, C. J., Jr & Rozeboom, L. E. (1964) *Indian J. med. Res.*, **52**, 703-709
Simmons, J. S., St. John, J. H. & Reynolds, F. H. K. (1931) *Philipp. J. Sci.*, **44**, 1-251
Toochinda, P. (1962) *The clinical course of Thai haemorrhagic fever.* In: *Symposium on Haemorrhagic Fever, 1961*, Bangkok, pp. 101-110 (SEATO Medical Research Monograph No. 2)
Wisseman, C. L., Jr, Sweet, B. H., Rosenzweig, E. C. & Eylar, O. R. (1963) *Amer. J. trop. Med. & Hyg.*, **12**, 620-623